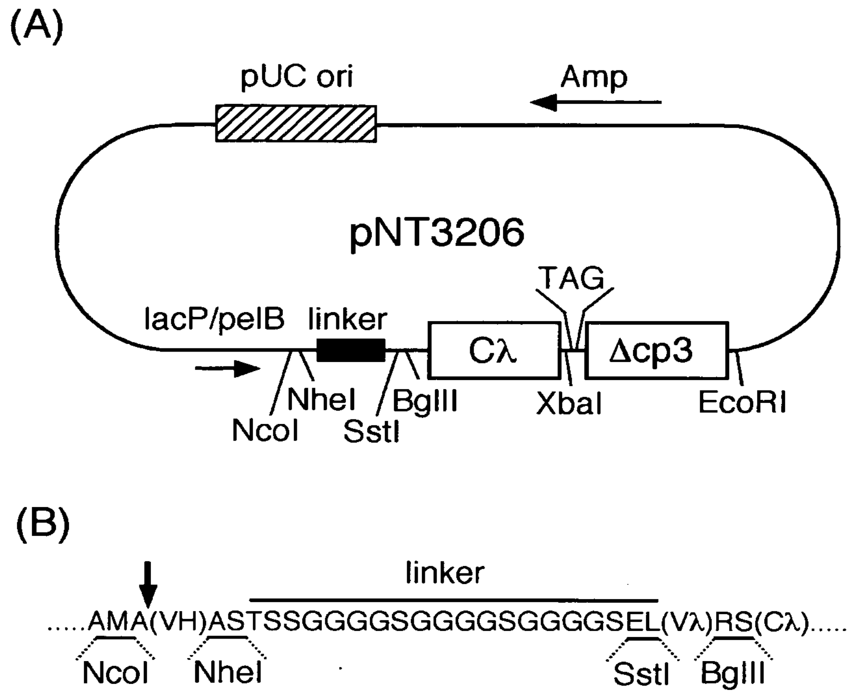

(A)
pUC ori
Amp
pNT3206
TAG
lacP/pelB
linker
Cλ
Δcp3
NheI
NcoI
SstI
BglII
XbaI
EcoRI
(B)
linker
.....AMA(VH)ASTSSGGGGSGGGGSGGGGSEL(Vλ)RS(Cλ).....
NcoI
NheI
SstI
BglII

FIG. 1

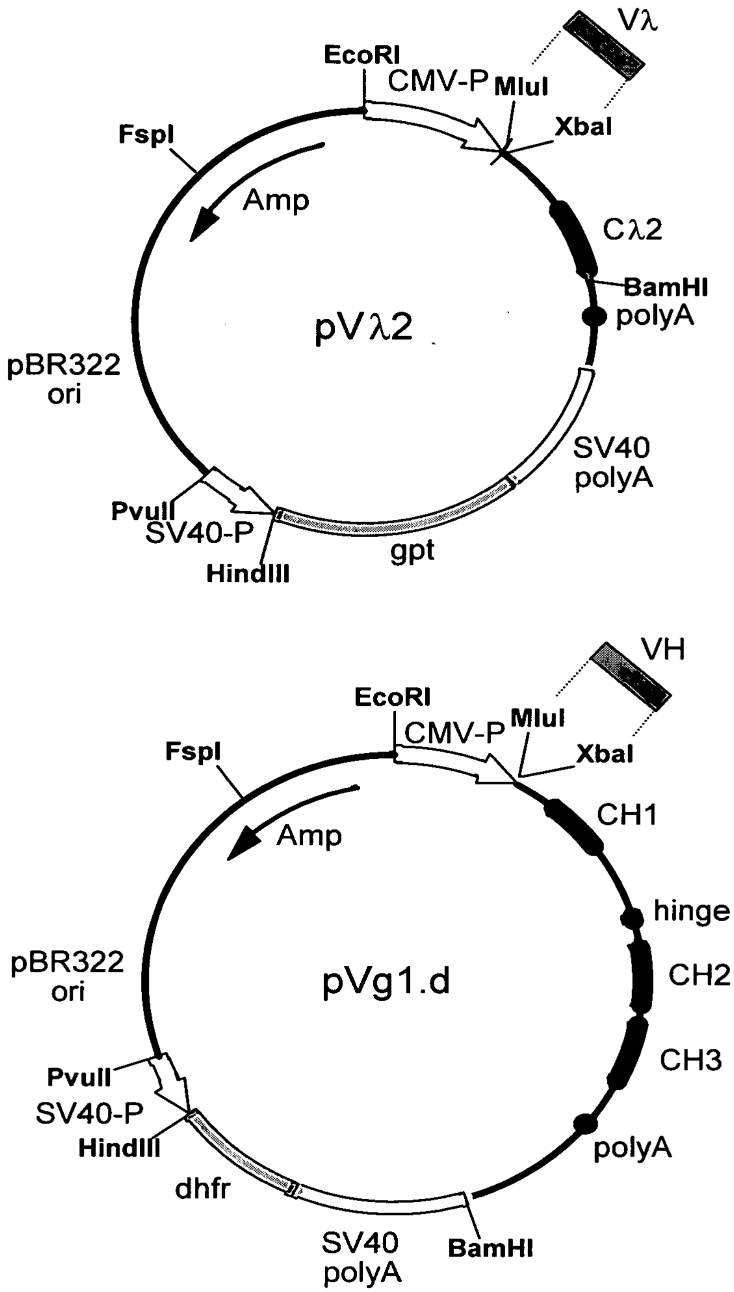
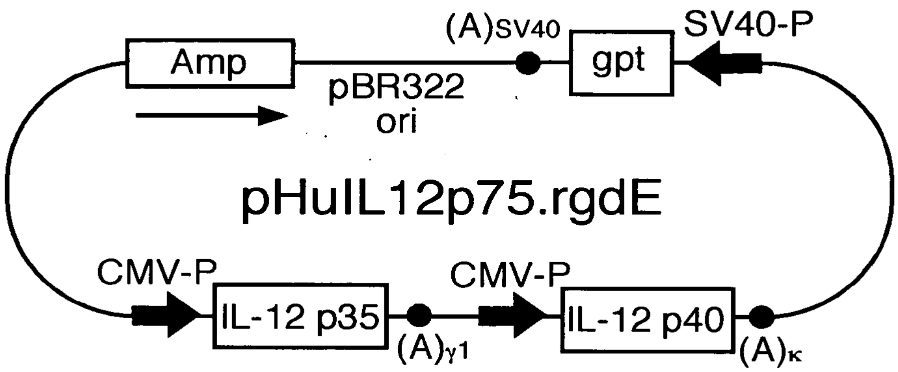

Vλ
EcoRI
CMV-P
MluI
XbaI
FspI
Amp
Cλ2
BamHI
polyA
pVλ2
pBR322
ori
SV40
polyA
PvuII
SV40-P
HindIII
gpt
VH
EcoRI
CMV-P
MluI
XbaI
FspI
Amp
CH1
hinge
pVg1.d
CH2
CH3
pBR322
ori
PvuII
SV40-P
HindIII
polyA
dhfr
SV40
polyA
BamHI

FIG. 2



(A)SV40
SV40-P
Amp
gpt
pBR322
ori
pHuIL12p75.rgdE
CMV-P
CMV-P
IL-12 p35
IL-12 p40
(A)γ1
(A)κ

FIG. 3

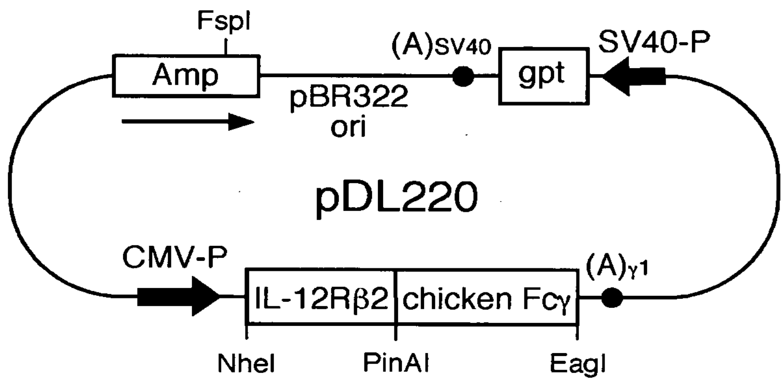

FspI
(A)SV40
SV40-P
Amp
gpt
pBR322
ori
pDL220
CMV-P
(A)γ1
IL-12Rβ2
chicken Fcγ
NheI
PinAI
EagI

FIG. 4

(A) Vλ sequences

```
                  1          2           3          4
         123456789 0123456789 01234567890 1234567899 0123456789
                                                  A
Chimeric B1     **ALTQPAS *VSANLGGTV KITCSGGYSG* *YYG WYQQKS PGSAPVTVIY
Humanized B1    SSELTQPPS *VSVALGQTV RITCSGGYSG* *YYGWYQQK* PGQAPVT VIY
DPL16           SSELTQDPA *VSVALGQTV RITC------- ----WYQQK* PGQAPVLVIY

         5          6          7          8          9
         0123456789 0123456789 0123456789 0123456789 01234556789
                                                         A
Chimeric B1     DNTRRPSDIP SRFSGSKSGS TATLTITGVQ ADDEAVYFCG TWDSSRVGI FG
Humanized B1    DNTRRPSDIP SRFSGSKSGS TATLTITGVQ AEDEADYYCG TWDSSRVGIFG
DPL16           -------GIP DRFSGSSSGN TASLTITGAQ AEDEADYYC- ---------FG

         1
         0
         01234567
Chimeric B1     AGTTLTVL
Humanized B1    GGTKLTVL
DPL16/Jλ2       GGTKLTVL
```

(B) VH sequences

```
                  1          2          3          4
         123456789 0123456789 0123456789 0123456789 0123456789
Chimeric B1     AVTLDESGG GLQTPGGALS LVCKASGFTF SSYSML WVRQ APGKGLEYVA
Humanized B1    EVQLVESGG GLVQPGGSLR LSCAASGFTF SSYSMLWVRQ APGKGLEY VA
DP-54           EVQLVESGG GLVQPGGSLR LSCAASGFTF S-----WVRQ APGKGLEWVA

         5           6          7          8                9
         01223456789 0123456789 0123456789 0122223456789
0123456789
           A                                 ABC
Chimeric B1     EITNTGRTRRY GAAVKG RATI SRDNGQSTVR LQLNNLRAEDTGT
YYCARSSVYS
Humanized B1    EITNTGRTRRY GAAVKGRA TI SRDNAKNTVY LQMNSLRAEDTAV
YYCARSSVYS
DP-54           ----------- ------RFTI SRDNAKNSLY LQMNSLRAEDTAV YYCAR--
---

         1                  1
         0                  1
         000000000123456789 0123
          ABCDEFGHI
Chimeric B1     CSYGWCAGNINAWGHGTEV IVSS
Humanized B1    CSYGWCAGNINAWGQGTLV TVSS
JH1             ------------WGQGTLV TVSS
```

FIG. 5

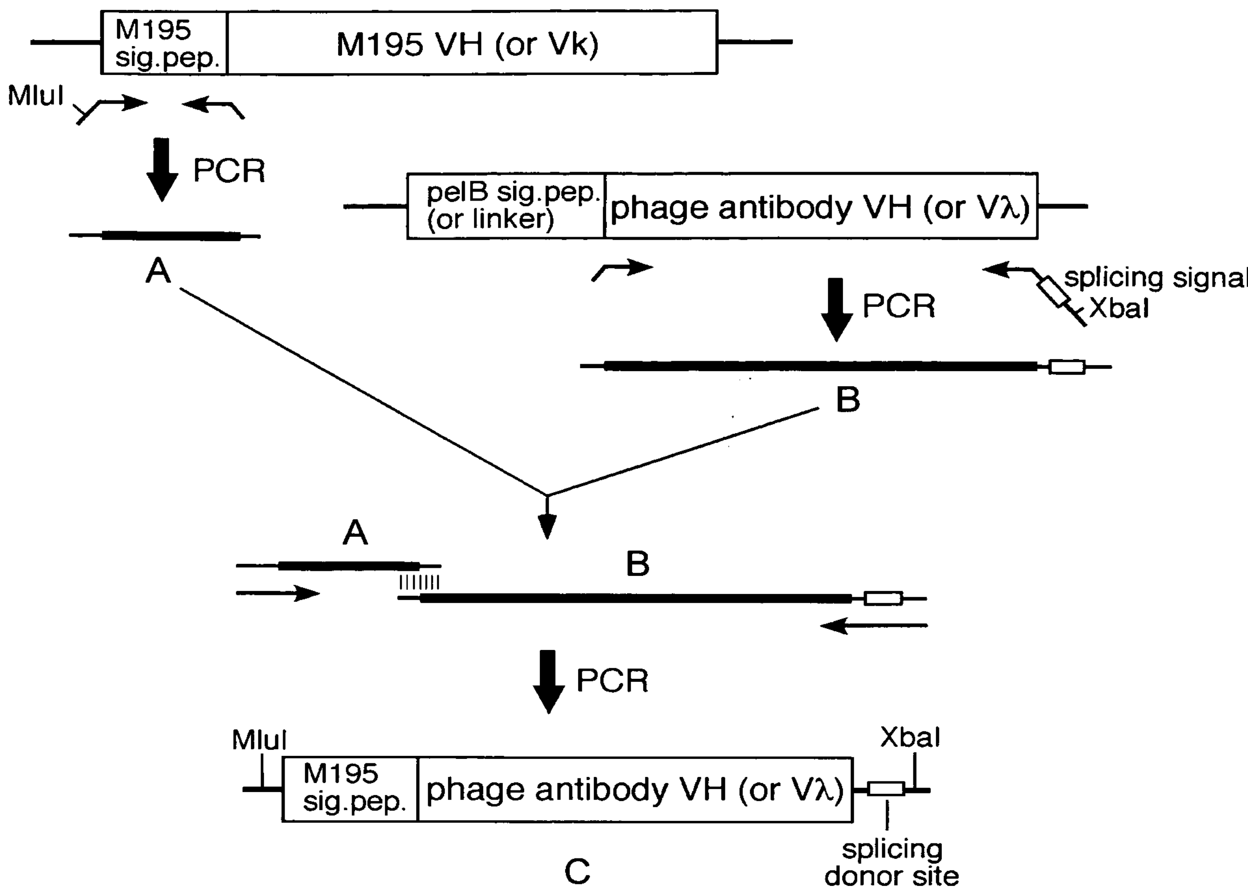

M195 sig.pep.
M195 VH (or Vk)
MluI
PCR
pelB sig.pep. (or linker)
phage antibody VH (or Vλ)
A
splicing signal
XbaI
PCR
B
A
B
PCR
MluI
XbaI
M195 sig.pep.
phage antibody VH (or Vλ)
splicing donor site
C

FIG. 6

(A) B1 Vλ mini exon

```
                                                30                             60
ACGCGTCTCGACCACCATGGAGAAAGACACACTCCTGCTATGGGTCCTACTTCTCTGGGT
                 M  E  K  D  T  L  L  L  W  V  L  L  L  W  V

                                                90                            120
TCCAGGTTCCACAGGTGCGCTGACTCAGCCGGCCTCAGTGTCAGCAAACCTGGGAGGAAC
  P  G  S  T  G  A  L  T  Q  P  A  S  V  S  A  N  L  G  G  T

                                               150                            180
CGTCAAGATCACCTGCTCCGGGGGTTACAGCGGCTATTATGGCTGGTACCAGCAGAAATC
  V  K  I  T  C  S  G  G  Y  S  G  Y  Y  G  W  Y  Q  Q  K  S

                                               180                            210
ACCTGGCAGTGCCCCTGTCACTGTGATCTATGACAACACCAGGAGACCCTCGGACATCCC
  P  G  S  A  P  V  T  V  I  Y  D  N  T  R  R  P  S  D  I  P

                                               240                            270
TTCACGATTCTCCGGTTCCAAATCCGGCTCCACAGCCACATTAACCATCACTGGGGTCCA
  S  R  F  S  G  S  K  S  G  S  T  A  T  L  T  I  T  G  V  Q

                                               300                            330
AGCCGACGACGAGGCTGTCTATTTCTGTGGGACCTGGGACAGCAGCCGTGTTGGTATATT
  A  D  D  E  A  V  Y  F  C  G  T  W  D  S  S  R  V  G  I  F

                                               360                  382
TGGGGCCGGGACAACCCTGACCGTCCTAAGTAAGTAGAATCCAAAGTCTAGA
  G  A  G  T  T  L  T  V  L
```

FIG. 7A

(B) B1 VH mini exon

```
                                        30                            60
ACGCGTCTCGACCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGC
                         M  G  W  S  W  I  F  L  F  L  L  S  G  T  A

                                        90                           120
TGGCGTCCACTCTGCCGTGACGTTGGACGAGTCTGGGGGCGGCCTCCAGACGCCCGGAGG
 G  V  H  S  A  V  T  L  D  E  S  G  G  G  L  Q  T  P  G  G

                                       150                           180
AGCGCTCAGCCTCGTCTGCAAGGCCTCCGGGTTCACCTTCAGTAGTTACAGCATGCTCTG
 A  L  S  L  V  C  K  A  S  G  F  T  F  S  S  Y  S  M  L  W

                                       210                           240
GGTGCGACAGGCGCCCGGCAAGGGGCTGGAATACGTCGCTGAAATTACCAACACTGGTAG
 V  R  Q  A  P  G  K  G  L  E  Y  V  A  E  I  T  N  T  G  R

                                       270                           300
GACCAGAAGATACGGGGCGGCGGTGAAGGGCCGTGCCACCATCTCGAGGGACAACGGGCA
 T  R  R  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  G  Q

                                       360                           390
GAGCACAGTGAGGCTGCAGCTGAACAACCTCAGGGCTGAGGACACCGGCACCTACTACTG
 S  T  V  R  L  Q  L  N  N  L  R  A  E  D  T  G  T  Y  Y  C

                                       420                           450
CGCCAGAAGTAGTGTTTATTCTTGTTCTTATGGTTGGTGTGCTGGTAACATCAACGCATG
 A  R  S  S  V  Y  S  C  S  Y  G  W  C  A  G  N  I  N  A  W

                                       480                  500
GGGCCACGGGACCGAAGTCATCGTCTCCTCCGGTAAGAATGGCGTCTAGA
 G  H  G  T  E  V  I  V  S  S
```

FIG. 7B

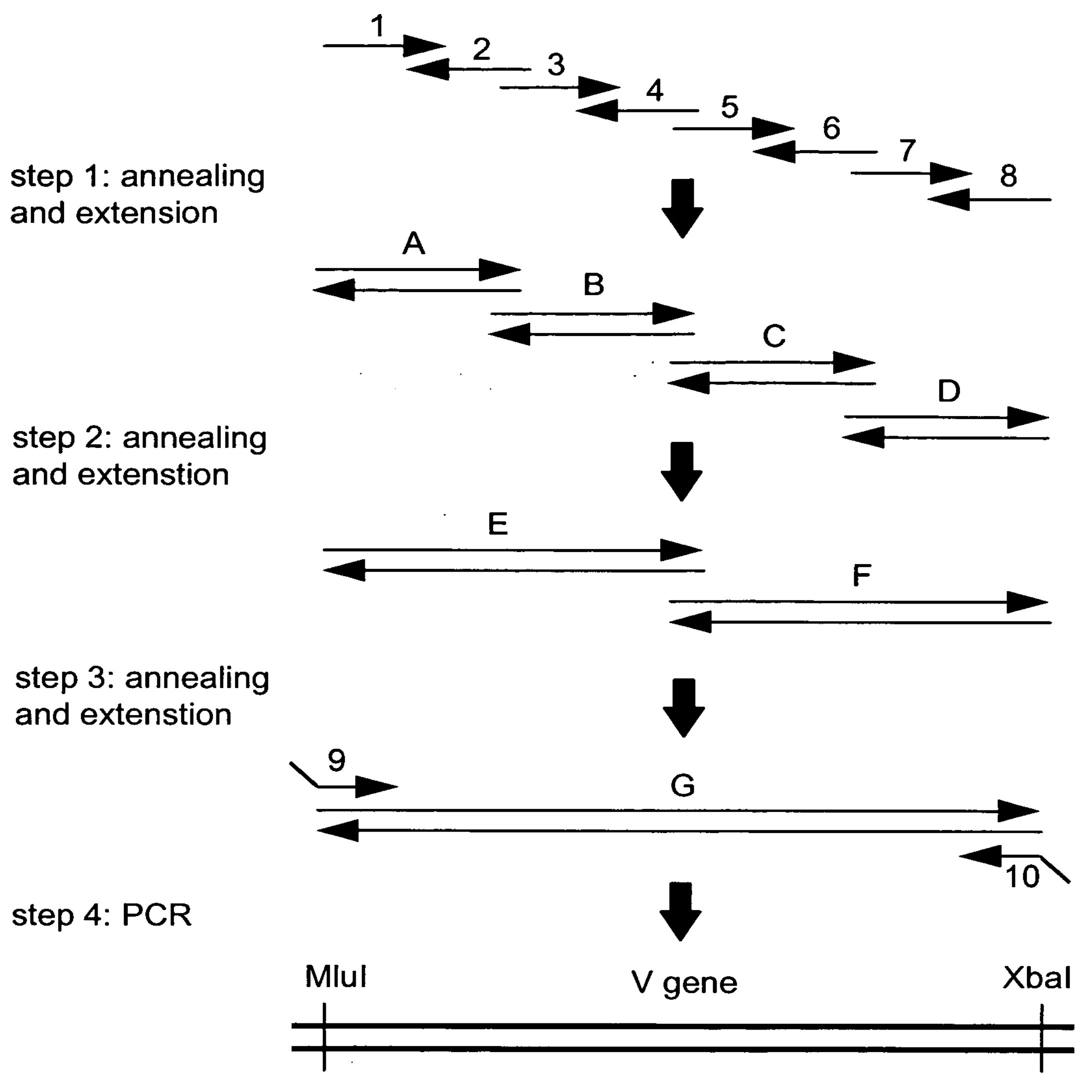

1
2
3
4
5
6
7
8
step 1: annealing and extension
A
B
C
D
step 2: annealing and extenstion
E
F
step 3: annealing and extenstion
9
G
10
step 4: PCR
MluI
V gene
XbaI

FIG. 8

(A) For HuB1 Vλ

Primer 1:
5'-CTAGCCACGCGTCCACCATGGAGAAAGACACACTCCTGCTATGGGTCCTACTTCTCTGGGTTCCAGGTTC-3'

Primer 2:
5'-CCAGGGCCACTGACACTGAAGGCGGCTGAGTCAGCTCAGAGCTACCTGTGGAACCTGGAACCCAGAGAAG-3'

Primer 3:
5'-CTTCAGTGTCAGTGGCCCTGGGACAGACCGTCAGGATCACCTGCTCCGGAGGTTACAGCGGCTATTATGGC-3'

Primer 4:
5'-GTTGTCATAAATCACAGTGACAGGAGCCTGGCCAGGTTTCTGCTGGTACCAGCCATAATAGCCGCTGTAAC-3'

Primer 5:
5'-CCTGTCACTGTGATTTATGACAACACCAGGAGACCCTCGGACATCCCTTCACGATTCTCCGGTTCCAAATCCG-3'

Primer 6:
5'-CCTCGTCCTCGGCTTGGACTCCAGTGATGGTTAATGTGGCTGTGGAGCCGGATTTGGAACCGGAGAATC-3'

Primer 7:
5'-GAGTCCAAGCCGAGGACGAGGCTGACTATTACTGTGGGACCTGGGACAGCAGCCGTGTTGGTATATTTGGAGG-3'

Primer 8:
5'-GACTCGTCTAGAGGGAGAAGAGACTCACCTAGGACGGTCAGCTTTGTCCCACCTCCAAATATACCAACACGGC-3'

Primer 9:
5'-CTAGCCACGCGTCCACCATG-3'

Primer 10:
5'-GACTCGTCTAGAGGGAGAAG-3'

(B) For HuB1 VH

Primer 1:
5'-CTAGCCACGCGTCCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCTGGCGTCCACTCTCAGG-3'

Primer 2:
5'-GAGCCTGAGGCTTCCTCCAGGCTGCACGAGTCCACCTCCGGACTCCACCAACTGCACCTGAGAGTGGACGCCAGCAG-3'

Primer 3:
5'-CCTGGAGGAAGCCTCAGGCTCAGCTGCGCCGCCTCCGGGTTCACCTTCAGTAGTTACAGCATGCTCTGGGTGCGACAGG-3'

Primer 4:
5'-CTTCTGGTCCTACCAGTGTTGGTAATTTCAGCGACGTATTCCAGTCCCTTGCCAGGCGCCTGTCGCACCCAGAGCATG-3'

Primer 5
5'-CCAACACTGGTAGGACCAGAAGATACGGAGCTGCGGTGAAGGGCCGTGCCACCATCTCTAGGGACAACGCCAAGAACAC-3'

Primer 6:
5'-GGCGCAGTAGTACACGGCGGTGTCCTCAGCCCTGAGGCTGTTCATCTGCAGGTACACTGTGTTCTTGGCGTTGTCCCTA-3'

Primer 7:
5'-CCGCCGTGTACTACTGCGCCAGAAGTAGTGTTTATTCTTGTTCTTATGGTTGGTGTGCTGGTAACATCAACGCATGG-3'

Primer 8:
5'-GACTCGTCTAGAGGTTGTGAGGACTCACCGGAGGAGACGGTGACCAGGGTTCCCTGGCCCCATGCGTTGATGTTACCAG-3'

Primer 9:
5'-CTAGCCACGCGTCCACCATG-3'

Primer 10:
5'-GACTCGTCTAGAGGTTGTGAG-3'

FIG. 9

(A) Vλ mini-exon of humanized B1

60
ACGCGTCCACCATGGAGAAAGACACACTCCTGCTATGGGTCCTACTTCTCTGGGTTCCAG
M E K D T L L L W V L L L W V P

120
GTTCCACAGGTAGCTCTGAGCTGACTCAGCCGCCTTCAGTGTCAGTGGCCCTGGGACAGA
G S T G S S E L T Q P P S V S V A L G Q

180
CCGTCAGGATCACCTGCTCCGGAGGTTACAGCGGCTATTATGGCTGGTACCAGCAGAAAC
T V R I T C S G G Y S G Y Y G W Y Q Q K

240
CTGGCCAGGCTCCTGTCACTGTGATTTATGACAACACCAGGAGACCCTCGGACATCCCTT
P G Q A P V T V I Y D N T R R P S D I P

300
CACGATTCTCCGGTTCCAAATCCGGCTCCACAGCCACATTAACCATCACTGGAGTCCAAG
S R F S G S K S G S T A T L T I T G V Q

360
CCGAGGACGAGGCTGACTATTACTGTGGGACCTGGGACAGCAGCCGTGTTGGTATATTTG
A E D E A D Y Y C G T W D S S R V G I F

409
GAGGTGGGACAAAGCTGACCGTCCTAG*GTGAGTCTCTTCTCCCTCTAGA*
G G G T K L T V L

FIG. 10A

(B) VH mini-exon of humanized B1

```
                                                            60
ACGCGTCCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCTGGCG
            M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G

                                                           120
TCCACTCTGAGGTGCAGTTGGTGGAGTCCGGAGGTGGACTCGTGCAGCCTGGAGGAAGCC
 V  H  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G  G  S

                                                           180
TCAGGCTCAGCTGCGCCGCCTCCGGGTTCACCTTCAGTAGTTACAGCATGCTCTGGGTGC
 L  R  L  S  C  A  A  S  G  F  T  F  S  S  Y  S  M  L  W  V

                                                           240
GACAGGCGCCTGGCAAGGGACTGGAATACGTCGCTGAAATTACCAACACTGGTAGGACCA
 R  Q  A  P  G  K  G  L  E  Y  V  A  E  I  T  N  T  G  R  T

                                                           300
GAAGATACGGAGCTGCGGTGAAGGGCCGTGCCACCATCTCGAGGGACAACGCCAAGAACA
 R  R  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  A  K  N

                                                           360
CAGTGTACCTGCAGATGAACAGCCTCAGGGCTGAGGACACCGCCGTGTACTACTGCGCCA
 T  V  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A

                                                           420
GAAGTAGTGTTTATTCTTGTTCTTATGGTTGGTGTGCTGGTAACATCAACGCATGGGGCC
 R  S  S  V  Y  S  C  S  Y  G  W  C  A  G  N  I  N  A  W  G

                                    469
AGGGAACCCTGGTCACCGTCTCCTCCGGTGAGTCCTCACAACCTCTAGA
 Q  G  T  L  V  T  V  S  S
```

FIG. 10B

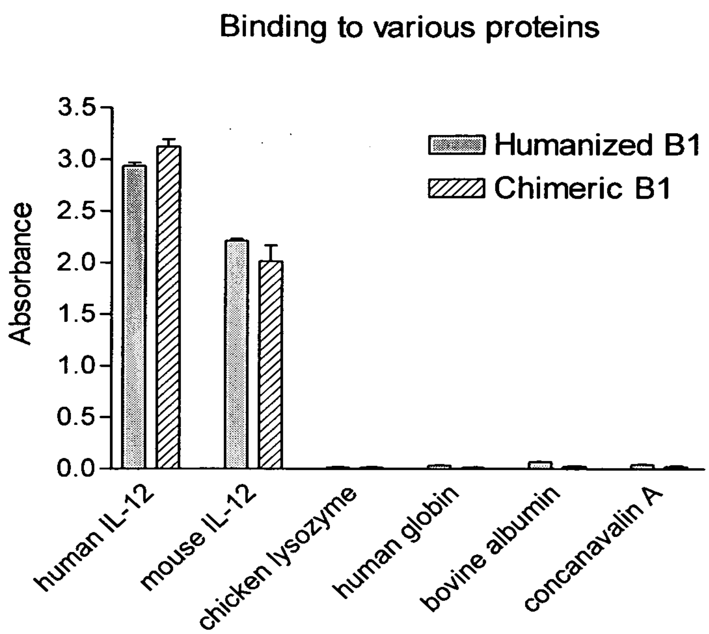
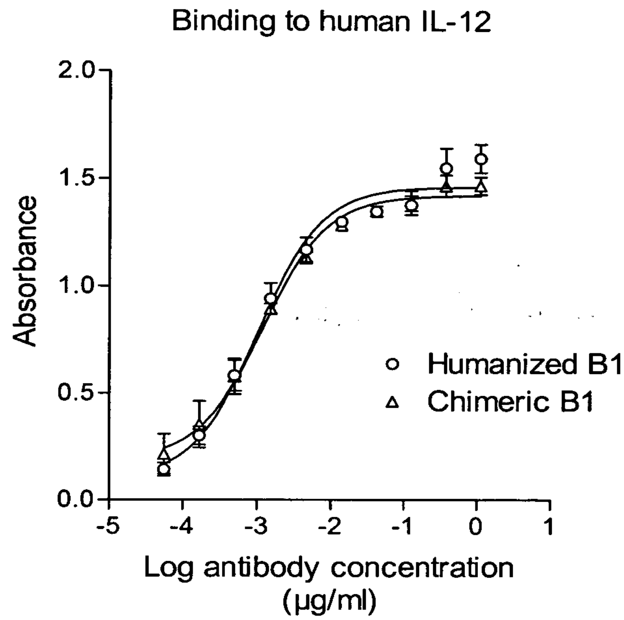

Binding to various proteins
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Absorbance
Humanized B1
Chimeric B1
human IL-12
mouse IL-12
chicken lysozyme
human globin
bovine albumin
concanavalin A

FIG. 11

(A)


Binding to human IL-12
2.0
1.5
1.0
0.5
0.0
Absorbance
-5
-4
-3
-2
-1
0
1
Log antibody concentration
(μg/ml)
Humanized B1
Chimeric B1

(B)

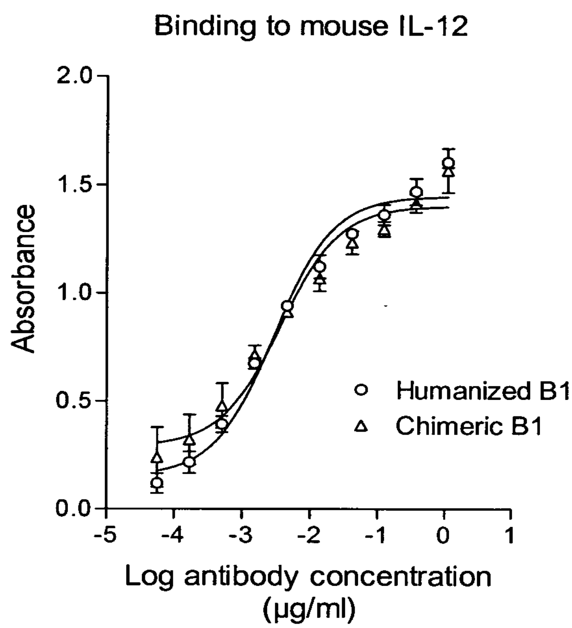
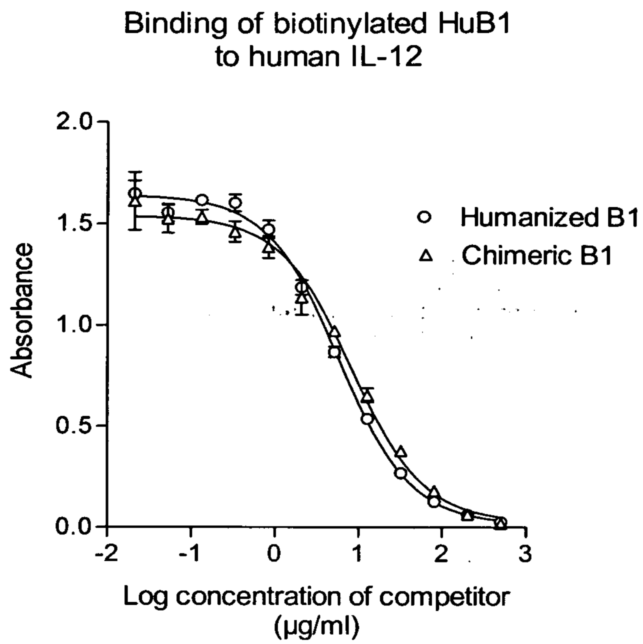

Binding to mouse IL-12
2.0
1.5
1.0
0.5
0.0
Absorbance
-5
-4
-3
-2
-1
0
1
Log antibody concentration
(μg/ml)
Humanized B1
Chimeric B1

FIG. 12

(A)


Binding of biotinylated HuB1
to human IL-12
Absorbance
2.0
1.5
1.0
0.5
0.0
-2
-1
0
1
2
3
Log concentration of competitor
(μg/ml)
Humanized B1
Chimeric B1

(B)

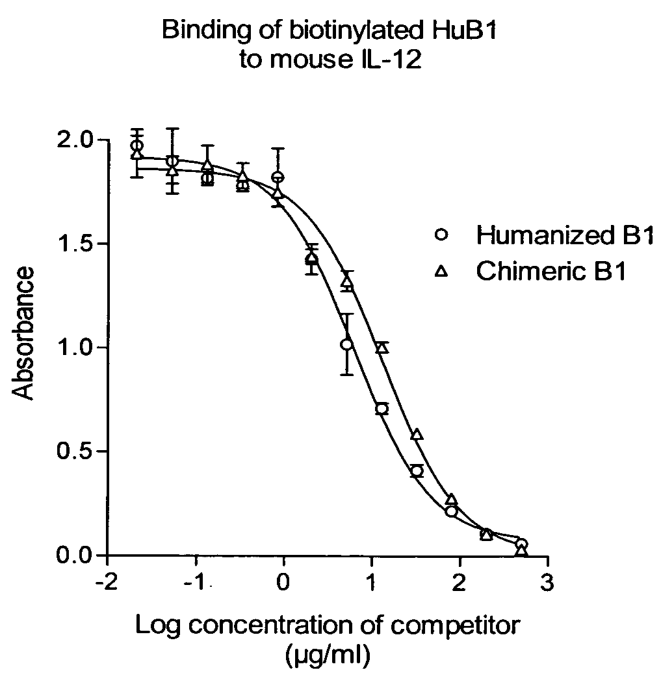

Binding of biotinylated HuB1
to mouse IL-12
Absorbance
2.0
1.5
1.0
0.5
0.0
-2
-1
0
1
2
3
Log concentration of competitor
(μg/ml)
Humanized B1
Chimeric B1

FIG. 13

[DD2 Vλ mini-exon]

```
                                                                  60
ACGCGTCTCGACCACCATGGAGAAAGACACACTCCTGCTATGGGTCCTACTTCTCTGGGT
                 M  E  K  D  T  L  L  L  W  V  L  L  L  W  V

                                                                 120
TCCAGGTTCCACAGGTGCGCTGACTCAGCCGGCCTCAGTGTCAGCAAACCCGGGAGAAAC
  P  G  S  T  G  A  L  T  Q  P  A  S  V  S  A  N  P  G  E  T

                                                                 180
CGTCAAGATCACCTGCCCCGGGGGTGGCATCTATGCTGGAAGGTACTATGGTTATGGCTG
  V  K  I  T  C  P  G  G  G  I  Y  A  G  R  Y  Y  G  Y  G  W

                                                                 240
GTTCCAGCAGAAGTCTCCTGGCAGTGCCCCTGTCACTGTGATCTATAGCAACGACAAGAG
  F  Q  Q  K  S  P  G  S  A  P  V  T  V  I  Y  S  N  D  K  R

                                                                 300
ACCCTCGGACATCCCTTCACGATTCTCCGGCTCCGCATCCGGCTCCACAGCCACATTAAC
  P  S  D  I  P  S  R  F  S  G  S  A  S  G  S  T  A  T  L  T

                                                                 360
CATCACTGGGGTCCAAGCCGACGACGAGGCTGTCTATTTCTGTGGGAGCCACGACAGCAA
  I  T  G  V  Q  A  D  D  E  A  V  Y  F  C  G  S  H  D  S  N

                                                                    423
TGTTGGTGTATTTGGGGCCGGGACAACCCTGACCGTCCTAAGTAAGTAGAATCCAAATCTAGA
  V  G  V  F  G  A  G  T  T  L  T  V  L
```

Fig. 14A

[DD2 VH mini exon]

```
                                                            60
ACGCGTCTCGACCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGC
                 M  G  W  S  W  I  F  L  F  L  L  S  G  T  A

                                                           120
TGGCGTCCACTCTGCCGTGACGTTGGACGAGTCCGGGGGCGGCCTCCAGACGCCCGGAGG
 G  V  H  S  A  V  T  L  D  E  S  G  G  G  L  Q  T  P  G  G

                                                           180
AGGGCTCAGCCTCGTCTGCAAGGCCTCCGGGTTCGACTTCAGCAACTATCAGTTGCAGTG
 G  L  S  L  V  C  K  A  S  G  F  D  F  S  N  Y  Q  L  Q  W

                                                           240
GGTGCGCCAGGCGCCCGGCAAGGGGCTGGAGTGGGTCGGTGGTATTGGCAGCAGTGGCAG
 V  R  Q  A  P  G  K  G  L  E  W  V  G  G  I  G  S  S  G  S

                                                           300
TAGCACATACTACGGGGCGGCGGTGAAGGGCCGTGCCACCATCTCGAGGGACAACGGGCA
 S  T  Y  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  G  Q

                                                           360
GAGCACAGTGAGACTGCAGCTGAACAACCTCAGGGCTGAGGACACCGGCACCTACTACTG
 S  T  V  R  L  Q  L  N  N  L  R  A  E  D  T  G  T  Y  Y  C

                                                           420
CACCAGAGGTGTTAGTCCTTACAGCTGTTGGTATGCCGGCCGTACTAGTTATACTTGTCA
 T  R  G  V  S  P  Y  S  C  W  Y  A  G  R  T  S  Y  T  C  H

                                                           480
TGCATATACTGCTGGTAGCATCGACGCATGGGGCCACGGGACCGAAGTCATCGTCTCCTC
 A  Y  T  A  G  S  I  D  A  W  G  H  G  T  E  V  I  V  S  S

                   500
CGGTAAGAATGGCGTCTAGA
```

Fig. 14B

(A) Vλ

```
                 1          2            3          4
        123456789 0123456789 0123456777789 01234567899 0123456789
                                  ABC               A
DD2     **ALTQPAS *VSANPGETV KITCPGGGIYAGR YYGYG WFQQKS PGSAPVTVIY
HuDD2   SSELTQDPA *VSVALGQTV RITCPGGGIYAGR YYGYGWF QQK* PGQAPVTVIY
DPL16   SSELTQDPA *VSVALGQTV RITC--------- -----WYQQK* PGQAPVLVIY
```

```
          5          6          7          8          9
          0123456789 0123456789 0123456789 0123456789 0123456789
DD2       SNDKRPSDIP SRFSGSASGS TATLTITGVQ ADDEAVYFCG SHDSNVGV FG
HuDD2     SNDKRPSDIP SRFSGSASGS TASLTITGAQ AEDEADYYCG SHDSNVGVFG
DPL16/Jλ2 -------GIP DRFSGSSSGN TASLTITGAQ AEDEADYYC- --------FG
```

```
        1
        0
        01234567
DD2     AGTTLTVL
HuDD2   GGTKLTVL
Jλ2     GGTKLTVL
```

(B) VH

```
                 1          2          3           4
        123456789 0123456789 0123456789 01234567899 0123456789
DD2     AVTLDESGG GLQTPGGGLS LVCKASGFDF SNYQLQ WVRQA PGKGLEWVGG
HuDD2   EVQLVESGG GLVQPGGSLR LSCAASGFD F SNYQLQWVRQA PGKGLEWVG G
DP-54   EVQLVESGG GLVQPGGSLR LSCAASGFTF S-----WVRQA PGKGLEWVA-
```

```
        5          6          7          8             9
        0123456789 0123456789 0123456789 0122223456789 0123456789
                                            ABC
DD2     IGSSGSSTYY GAAVKGRATI SRDNGQSTVR LQLNNLRAEDTGT YYCTRGVSPY
HuDD2   IGSSGSSTYY GAAVKGRA TI SRDNAKNSVY LQMNSLRAEDTAV YYCT RGVSPY
DP-54   ---------- ------RFTI SRDNAKNSLY LQMNSLRAEDTAV YYCAR-----
```

```
        1
        0
        0000000000123456789 01234567890123
         ABCDEFGHI
DD2     SCWYAGRTSYTCHAYTAGS IDA WGHGTEVIVSS
HuDD2   SCWYAGRTSYTCHAYTAGS IDAWGQGTLVTVSS
JH1     ------------------- ---WGQGTLVTVSS
```

Fig. 15

(A) For HuDD2 Vλ

Primer 1
5'-ACGCGTCCACCATGGAGAAAGACACACTCCTGCTGTGGGTCCTACTTCTCTGGGTTCCAGGTTCCACAGGTTC-3'

Primer 2
5'-CCTGACTGTCTGTCCCAAGGCCACAGACACAGCAGGGTCCTGAGTCAGCTCAGAAGAACCTGTGGAACCTGGAAC-3'

Primer 3
5'-CCTTGGGACAGACAGTCAGGATCACATGCCCCGGAGGTGGCATCTATGCTGGACGCTACTATGGTTATGGCTG-3'

Primer 4
5'-CGTTGCTATAGATGACAGTTACAGGGGCCTGTCCTGGCTTCTGCTGGAACCAGCCATAACCATAGTAGCG-3'

Primer 5
5'-CTGTAACTGTCATCTATAGCAACGACAAGAGACCCTCGGACATCCCTTCACGATTCTCTGGCTCCGCATC-3'

Primer 6
5'-CATCTTCCGCCTGAGCCCCAGTGATGGTCAAGGAAGCTGTGGAGCCTGATGCGGAGCCAGAGAATCGTG-3'

Primer 7
5'-GGCTCAGGCGGAAGATGAGGCTGACTATTACTGTGGGAGCCACGACAGCAATGTTGGTGTATTTGG-3'

Primer 8
5'-TCTAGAGGGAGAAGAGACTCACCTAGGACGGTCAGCTTTGTCCCACCGCCAAATACACCAACATTGCTGTC-3'

Primer 9
5'-CTACGAACGCGTCCACCATGGAGAAAG-3'

Primer 10
5'-GACTTCTCTAGAGGGAGAAGAGACTCACC-3'

(B) For HuDD2 VH

Primer 1
5'-ACGCGTCCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCTGGCGTGCACTCTGAGGTGCAGCTG-3'

Primer 2
5'-GGCTGCACAGGAGAGTCTCAGGGACCCCCCAGGCTGGACCAAGCCTCCCCCAGACTCCACCAGCTGCACCTCAGAGTGCA-3'

Primer 3
5'-TGAGACTCTCCTGTGCAGCCTCTGGATTCGACTTTAGTAACTATCAGTTGCAGTGGGTCCGCCAGGCTCCAGGGAAGGGG-3'

Primer 4
5'-AACCGCAGCTCCGTAGTATGTGCTACTGCCACTGCTGCCAATACCACCCACCCACTCCAGCCCCTTCCCTGGAGCCTGGC-3'

Primer 5
5'-CATACTACGGAGCTGCGGTTAAGGGCCGAGCCACCATCTCCAGAGACAACGCCAAGAACTCAGTGTATCTGCAAATGAAC-3'

Primer 6
5'-CTGTAAGGACTAACACCTCTGGTACAGTAATACACAGCCGTGTCCTCGGCTCTCAGGCTGTTCATTTGCAGATACACTGA-3'

Primer 7
5'-AGAGGTGTTAGTCCTTACAGCTGTTGGTATGCCGGCCGTACTAGTTATACTTGTCATGCATATACTGCTGGTAGCATCGA-3'

Primer 8
5'-TCTAGAAGTACAGCAGACTCACCTGAGGAGACGGTGACCAGGGTTCCCTGGCCCCATGCGTCGATGCTACCAGCAGTATA-3'

Primer 9
5'-CTACGAACGCGTCCACCATGGGATGG-3'

Primer 10
5'-GACTTCTCTAGAAGTACAGCAGACTCAC-3'

Fig. 16

[HuDD2 Vλ mini exon]

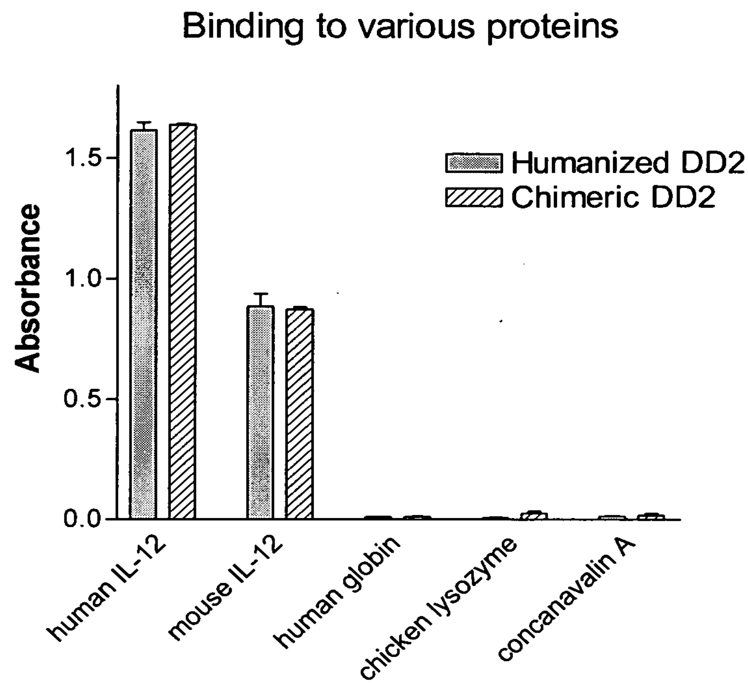
```
                                                             60
ACGCGTCCACCATGGAGAAAGACACACTCCTGCTGTGGGTCCTACTTCTCTGGGTTCCAG
           M  E  K  D  T  L  L  L  W  V  L  L  L  W  V  P

                                                            120
GTTCCACAGGTTCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGA
G  S  T  G  S  S  E  L  T  Q  D  P  A  V  S  V  A  L  G  Q

                                                            180
CAGTCAGGATCACATGCCCCGGAGGTGGCATCTATGCTGGACGCTACTATGGTTATGGCT
T  V  R  I  T  C  P  G  G  G  I  Y  A  G  R  Y  Y  G  Y  G

                                                            240
GGTTCCAGCAGAAGCCAGGACAGGCCCCTGTAACTGTCATCTATAGCAACGACAAGAGAC
W  F  Q  Q  K  P  G  Q  A  P  V  T  V  I  Y  S  N  D  K  R

                                                            300
CCTCGGACATCCCTTCACGATTCTCTGGCTCCGCATCAGGCTCCACAGCTTCCTTGACCA
P  S  D  I  P  S  R  F  S  G  S  A  S  G  S  T  A  S  L  T

                                                            360
TCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACTGTGGGAGCCACGACAGCAATG
I  T  G  A  Q  A  E  D  E  A  D  Y  Y  C  G  S  H  D  S  N

                                                              421
TTGGTGTATTTGGCGGTGGGACAAAGCTGACCGTCCTAGGTGAGTCTCTTCTCCCTCTAGA
V  G  V  F  G  G  G  T  K  L  T  V  L
```

Fig. 17A

[HuDD2 VH mini exon]

```
                                                                      60
ACGCGTCCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCTGGCG
            M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G

                                                                     120
TGCACTCTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCC
V  H  S  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G  G  S

                                                                     180
TGAGACTCTCCTGTGCAGCCTCTGGATTCGACTTTAGTAACTATCAGTTGCAGTGGGTCC
L  R  L  S  C  A  A  S  G  F  D  F  S  N  Y  Q  L  Q  W  V

                                                                     240
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGGTGGTATTGGCAGCAGTGGCAGTAGCA
R  Q  A  P  G  K  G  L  E  W  V  G  G  I  G  S  S  G  S  S

                                                                     300
CATACTACGGAGCTGCGGTTAAGGGCCGAGCCACCATCTCCAGAGACAACGCCAAGAACT
T  Y  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  A  K  N

                                                                     360
CAGTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTACCA
S  V  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  T

                                                                     420
GAGGTGTTAGTCCTTACAGCTGTTGGTATGCCGGCCGTACTAGTTATACTTGTCATGCAT
R  G  V  S  P  Y  S  C  W  Y  A  G  R  T  S  Y  T  C  H  A

                                                                     480
ATACTGCTGGTAGCATCGACGCATGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTG
Y  T  A  G  S  I  D  A  W  G  Q  G  T  L  V  T  V  S  S

                    499
AGTCTGCTGTACTTCTAGA
```

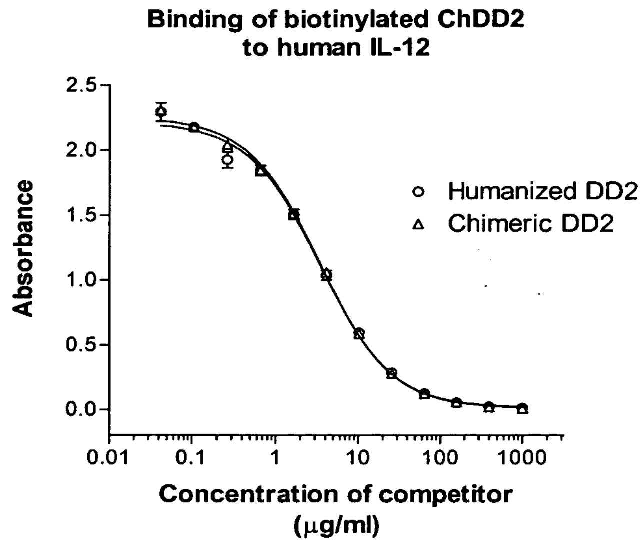
Fig. 17B



Binding to various proteins
Humanized DD2
Chimeric DD2
Absorbance
1.5
1.0
0.5
0.0
human IL-12
mouse IL-12
human globin
chicken lysozyme
concanavalin A

Fig. 18

(A)


Binding of biotinylated ChDD2
to human IL-12
Absorbance
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
Concentration of competitor
(μg/ml)
Humanized DD2
Chimeric DD2

(B)

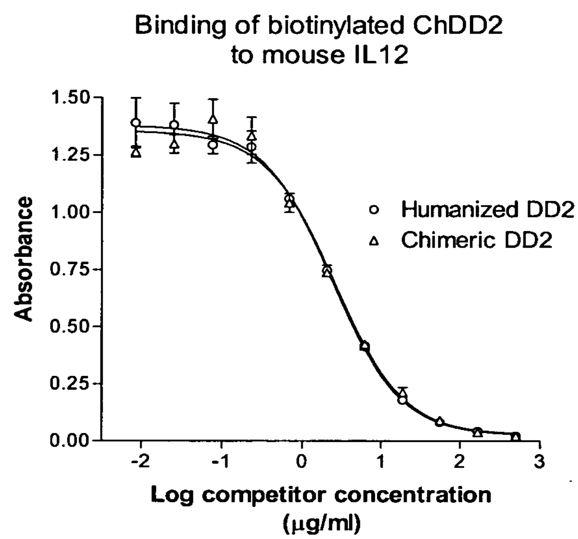
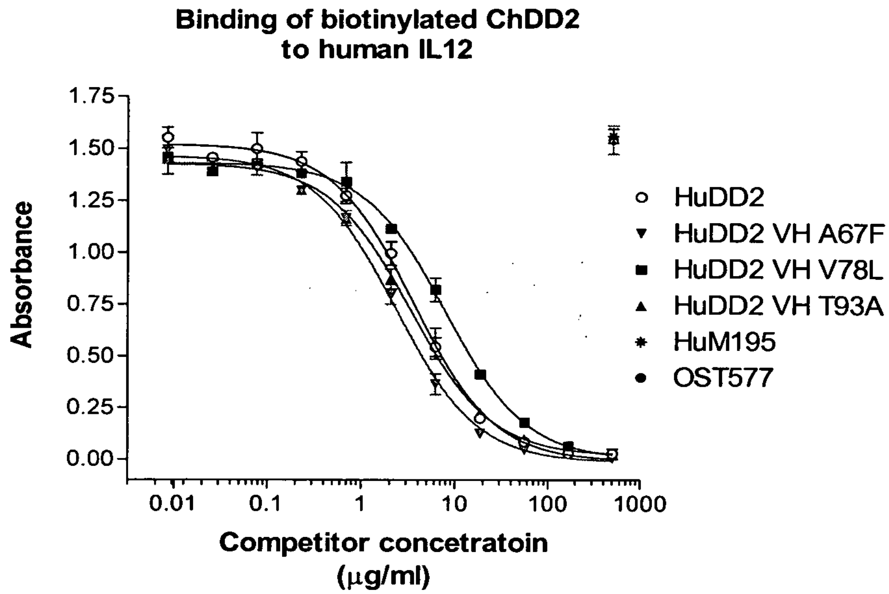

Binding of biotinylated ChDD2
to mouse IL12
Absorbance
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-2
-1
0
1
2
3
Log competitor concentration
(μg/ml)
Humanized DD2
Chimeric DD2

Fig. 19

(A)


Binding of biotinylated ChDD2
to human IL12
Absorbance
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.01
0.1
1
10
100
1000
Competitor concetratoin
(μg/ml)
HuDD2
HuDD2 VH A67F
HuDD2 VH V78L
HuDD2 VH T93A
HuM195
OST577

(B)

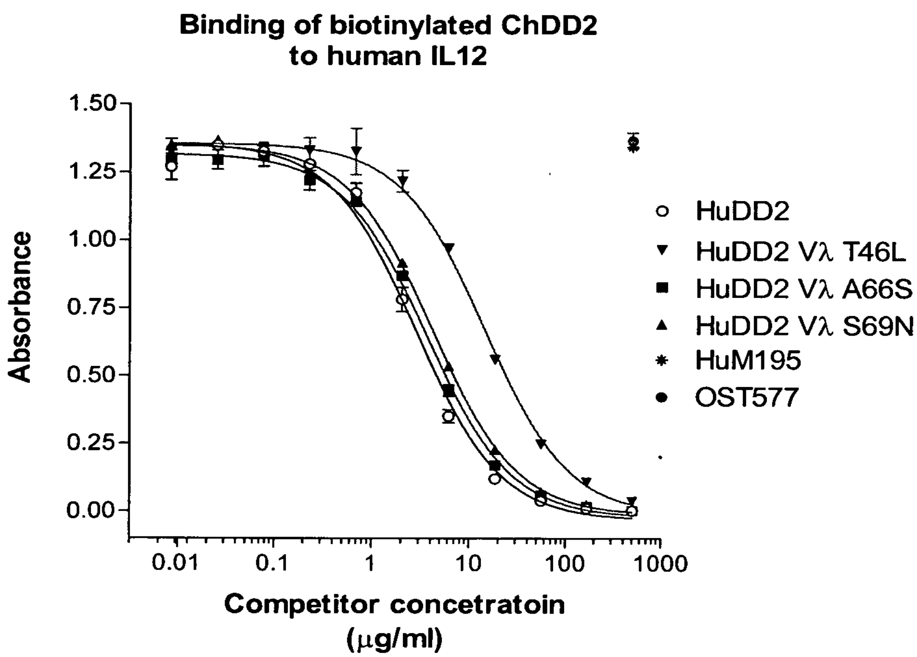

Binding of biotinylated ChDD2
to human IL12
Absorbance
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.01
0.1
1
10
100
1000
Competitor concetratoin
(μg/ml)
HuDD2
HuDD2 Vλ T46L
HuDD2 Vλ A66S
HuDD2 Vλ S69N
HuM195
OST577

Fig. 20

[D3 Vλ mini-exon]

```
                                                          60
ACGCGTCTCGACCACCATGGAGAAAGACACACTCCTGCTATGGGTCCTACTTCTCTGGGT
                  M  E  K  D  T  L  L  L  W  V  L  L  L  W  V

                                                         120
TCCAGGTTCCACAGGTGCGCTGACTCAGCCGGCCTCGGTGTCAGCAAACCCAGGAGAAAC
  P  G  S  T  G  A  L  T  Q  P  A  S  V  S  A  N  P  G  E  T

                                                         180
CGTCAAGATCACCTGCTCCGGGGGTAGCTACTATGGCTGGTACCAGCAGAAGTCTCCTGG
  V  K  I  T  C  S  G  G  S  Y  Y  G  W  Y  Q  Q  K  S  P  G

                                                         240
CAGTGCCCCTGTCACTGTGATTTATGACAACGACAAGAGACCCTCGGACATCCCTTCACG
  S  A  P  V  T  V  I  Y  D  N  D  K  R  P  S  D  I  P  S  R

                                                         300
ATTCTCCGGTTCCAAATCCGGCTCCACGGGCACATTAACCATCACTGGGGTCCAAGCCGA
  F  S  G  S  K  S  G  S  T  G  T  L  T  I  T  G  V  Q  A  E

                                                         360
GGATGAGGCTGTCTATTTCTGTGGGAGTGCAGACAGCGCCTATGTTGGTATATTTGGGGC
  D  E  A  V  Y  F  C  G  S  A  D  S  A  Y  V  G  I  F  G  A

                                         406
CGGGACAACCCTGACCGTCCTAAGTAAGTAGAATCCAAAGTCTAGA
  G  T  T  L  T  V  L
```

Fig. 21A

[D3 VH mini-exon]

```
                                                                      60
ACGCGTCTCGACCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGC
                   M  G  W  S  W  I  F  L  F  L  L  S  G  T  A

                                                                     120
TGGCGTCCACTCTGCCGTGACGTTGGACGAGTCCGGGGGCGGCCTCCAGACGCCCGGAGG
  G  V  H  S  A  V  T  L  D  E  S  G  G  G  L  Q  T  P  G  G

                                                                     180
AGCGCTCAGCCTCGTCTGCAGGGCCTCCGGGTTCTCTATCGGCAGTTACAACATGCACTG
  A  L  S  L  V  C  R  A  S  G  F  S  I  G  S  Y  N  M  H  W

                                                                     240
GGTGCGACAGGCGCCCGGCAAGGGGCTGGAGTGGGTCGCTGGTATTAGCGGTGCTGGTAG
  V  R  Q  A  P  G  K  G  L  E  W  V  A  G  I  S  G  A  G  S

                                                                     300
TCGCACAGCATGGTACGGGGCGGCGGTGAAGGGCCGTGCCACCATCTCGAGGGACAACGG
  R  T  A  W  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  G

                                                                     360
GCAGAGCACAGTGAGGCTGCAGCTGAACAACCTCAGGGCCGAGGACACCGGCACCTACTA
  Q  S  T  V  R  L  Q  L  N  N  L  R  A  E  D  T  G  T  Y  Y

                                                                     420
CTGCGCCAAAGACTATGGTGGTAGTGGTTCCCCATGGTATGGTTGGGGTGCTGCTAGTTG
  C  A  K  D  Y  G  G  S  G  S  P  W  Y  G  W  G  A  A  S  W

                                                                       482
GATCGACGCATGGGGCCACGGGACCGAAGTCATCGTCTCCTCCGGTAAGAATGGCGTCTAGA
  I  D  A  W  G  H  G  T  E  V  I  V  S  S
```

Fig. 21B

[Vλ]

```
                          1          2          3           4
               123456789 0123456789 0123456789 01234567899 0123456789
                                                       A
Chicken D3     **ALTQPAS *VSANPGETV KITCSGGS**  **YYGWYQQKS PGSAPVTVIY
Humanized D3   SSELTQDPA *VSVALGQTV RITCSGGS** **YYGWYQQK* PGQAPVT_VIY
3-23OIIIB237   SSELTQDPA *VSVALGQTV RITC--------- --WYQQK* PGQAPVLVIY

               5          6          7          8          9
               0123456789 0123456789 0123456789 0123456789 01234556789
                                                                A
Chicken D3     DNDKRPSDIP SRFSGSKSGS TGTLTITGVQ AEDEAVYFCG_ SADSAYVGIFG
Humanized D3   DNDKRPSDIP SRFSGSKSGS TGSLTITGAQ AEDEADYYCG SADSAYVGIFG
3-23OIIIB237/Jλ2 -------GIP DRFSGSSSGN TASLTITGAQ AEDEADYYC- ---------FG

               1
               0
               01234567
Chicken D3     AGTTLTVL
Humanized D3   GGTKLTVL
Jλ2            GGTKLTVL
```

[VH]

```
                          1          2          3          4
               123456789 0123456789 0123456789 0123456789 0123456789
Chicken D3     AVTLDESGG GLQTPGGALS LVCRASGFSI GSYNMH WVRQ APGKGLEWVA
Humanized D3   EVQLLESGG GLVQPGGSLR LSCAASGFSI_ GSYNMHWVRQ APGKGLEWVA_
ha316          EVQLLESGG GLVQPGGSLR LSCAASGFTF S-----WVRQ APGKGLEWVS

               5            6          7          8             9
               012223456789 0123456789 0123456789 0122223456789 0123456789
                 AB                                  ABC
Chicken D3     GISGAGSRTAWY GAAVKG RATI SRDNGQSTVR LQLNNLRAEDTGT YYCAKDYGGS
Humanized D3   GISGAGSRTAWY GAAVKGRA_TI SRDNAKNTVY LQMNSLRAEDTAV YYCAKDYGGS
ha316          ----------- -------RFTI SRDNSKNTLY LQMNSLRAEDTAV YYCAK-----

               1                     1
               0                     1
               0000000000000123456789 0123
                ABCDEFGHIJKL
Chicken D3     GSPWYGWGAASWIDA WGHGTEV IVSS
Humanized D3   GSPWYGWGAASWIDAWGQGTLV TVSS
ha316          ---------------WGQGTLV TVSS
```

Fig. 22

[HuD3 Vλ mini-exon]

```
                                                            60
ACGCGTCCACCATGGAGAAAGACACACTCCTGCTGTGGGTCCTACTTCTCTGGGTTCCAG
           M  E  K  D  T  L  L  L  W  V  L  L  L  W  V  P

                                                           120
GTTCCACAGGTTCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGA
G  S  T  G  S  S  E  L  T  Q  D  P  A  V  S  V  A  L  G  Q

                                                           180
CAGTCAGGATCACATGCTCCGGGGGTAGCTACTATGGCTGGTACCAGCAGAAGCCAGGAC
T  V  R  I  T  C  S  G  G  S  Y  Y  G  W  Y  Q  Q  K  P  G

                                                           240
AGGCCCCTGTAACTGTCATCTATGACAACGACAAGAGACCCTCGGACATCCCTTCACGAT
Q  A  P  V  T  V  I  Y  D  N  D  K  R  P  S  D  I  P  S  R

                                                           300
TCTCTGGCTCCAAATCAGGCTCCACAGGCTCCTTGACCATCACTGGGGCTCAGGCGGAAG
F  S  G  S  K  S  G  S  T  G  S  L  T  I  T  G  A  Q  A  E

                                                           360
ATGAGGCTGACTATTACTGTGGGAGTGCAGACAGCGCCTATGTTGGTATATTTGGCGGTG
D  E  A  D  Y  Y  C  G  S  A  D  S  A  Y  V  G  I  F  G  G

                                          403
GGACAAAGCTGACCGTCCTAGGTGAGTCTCTTCTCCCTCTAGA
G  T  K  L  T  V  L
```

Fig. 23A

[HuD3 VH mini-exon]

```
                                                                   60
ACGCGTCCACCATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCTGGCG
            M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G

                                                                  120
TGCACTCTGAGGTGCAGCTGCTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCC
V  H  S  E  V  Q  L  L  E  S  G  G  G  L  V  Q  P  G  G  S

                                                                  180
TGAGACTCTCCTGTGCAGCCTCTGGATTCTCTATCGGCAGTTACAACATGCACTGGGTCC
L  R  L  S  C  A  A  S  G  F  S  I  G  S  Y  N  M  H  W  V

                                                                  240
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCTGGTATTAGCGGTGCTGGTAGTCGCA
R  Q  A  P  G  K  G  L  E  W  V  A  G  I  S  G  A  G  S  R

                                                                  300
CAGCATGGTACGGGGCGGCGGTGAAGGGCCGAGCCACCATCTCCAGAGACAACGCCAAGA
T  A  W  Y  G  A  A  V  K  G  R  A  T  I  S  R  D  N  A  K

                                                                  360
ACACAGTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTG
N  T  V  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C

                                                                  420
CCAAAGACTATGGTGGTAGTGGTTCCCCATGGTATGGTTGGGGTGCTGCTAGTTGGATCG
A  K  D  Y  G  G  S  G  S  P  W  Y  G  W  G  A  A  S  W  I

                                                                  481
ACGCATGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGAGTCTGCTGTACTTCTAGA
D  A  W  G  Q  G  T  L  V  T  V  S  S
```

Fig. 23B

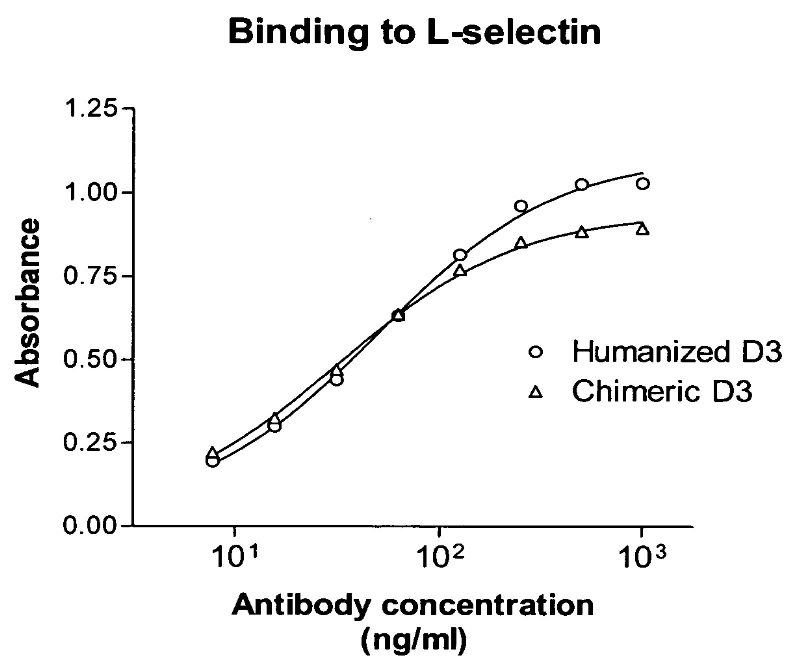

Binding to L-selectin
1.25
1.00
0.75
0.50
0.25
0.00
Absorbance
Humanized D3
Chimeric D3
10^1
10^2
10^3
Antibody concentration
(ng/ml)

Fig. 24

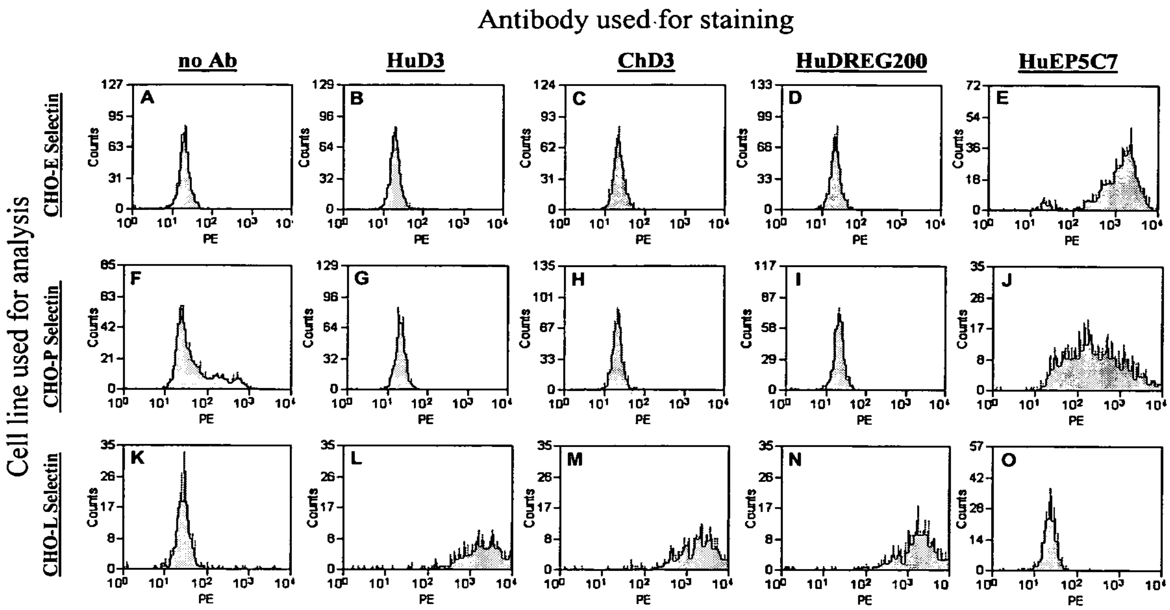
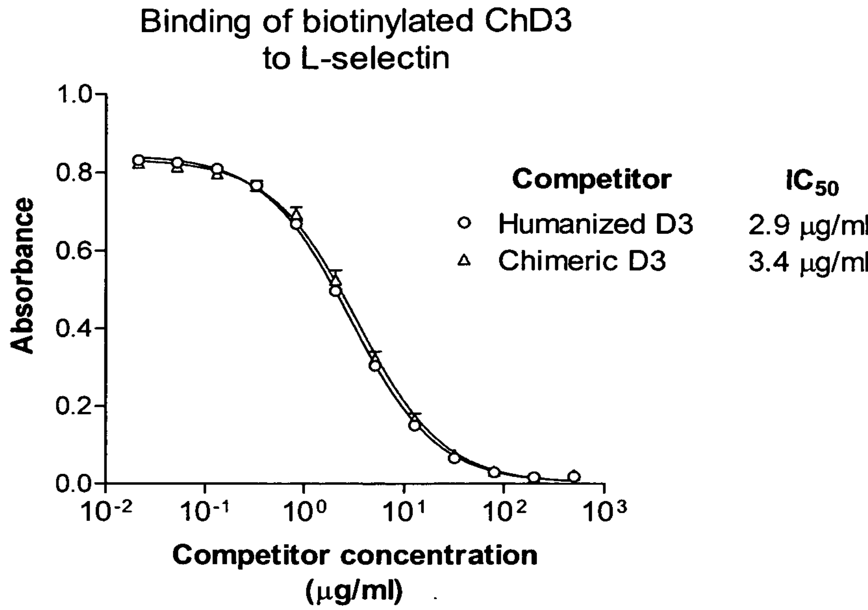

Antibody used for staining
no Ab
HuD3
ChD3
HuDREG200
HuEP5C7
Cell line used for analysis
CHO-E Selectin
CHO-P Selectin
CHO-L Selectin
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Counts
PE

Fig. 25

(A)


Binding of biotinylated ChD3
to L-selectin
Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Competitor concentration
(μg/ml)
Competitor
IC_{50}
Humanized D3 2.9 μg/ml
Chimeric D3 3.4 μg/ml

(B)

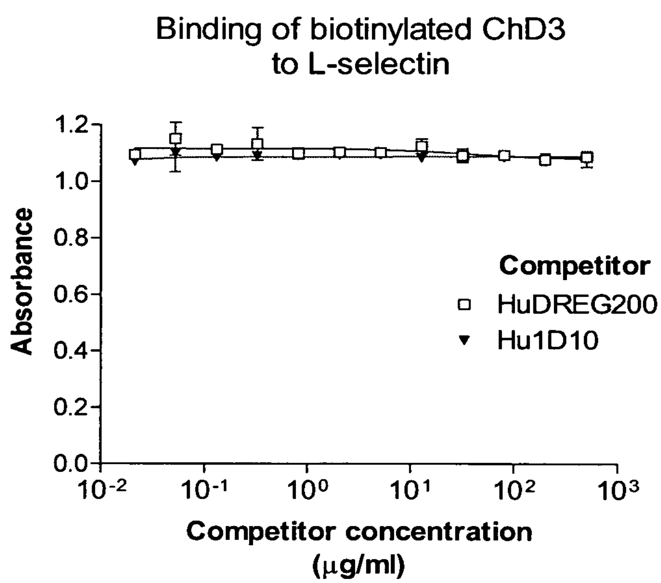

Binding of biotinylated ChD3
to L-selectin
Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Competitor concentration
(μg/ml)
Competitor
HuDREG200
Hu1D10

Fig. 26